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EFFECTS OF HYDRAZINE ON PREGNANT ICR MICE

ROBERT D. LYNG
WILLIAM C. KELLER
KENNETH C. BACK

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AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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* Indiana University-Purdue University
at Fort Wayne
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Fort Wayne, Indiana 46805

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As more women enter jobs formerly held by men there is a risk that exposure to some chemicals may cause birth defects in an unborn child. To determine the teratogenic potential of a chemical, it is frequently necessary to test the compound in more than one animal species. The objective of this project was to determine the usefulness of ICR Mice as a test species. Hydrazine was injected intraperitoneally on days 6, 7, 8, and 9 of gestation at concentrations to 4, 12, 20, 30, and 40 mg/kg body weight. Physiological saline was administered

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in the same manner to a control group. Hydrazine in concentrations up to 20 mg/kg body weight had no significant effect on the number of implantations per female, the mean number of viable fetuses per litter, or the mean number of resorptions per litter. At concentrations of 30 and 40 mg/kg, hydrazine was fetotoxic and 4 of 21 females died at a concentration of 40 mg/kg. As the dose was increased from 4 to 30 mg/kg there was an increasing percentage of litters with soft tissue anomalies with exencephaly and hydronephrosis produced most often. Skeletal abnormalities were produced at a higher frequency and at lower concentrations than soft tissue abnormalities. Supernumerary ribs accounted for most of these defects. Pregnant mice receiving 12 and 20 mg/kg showed a lower rate of weight gain during the injection period than those receiving saline or 4 mg/kg. After the injection period, from days 9 through 11, the mice receiving 12 and 20 mg/kg had the highest rate of gain.

PREFACE

This research was performed in the Toxicology Branch, Toxic Hazards Division, Aerospace Medical Research Laboratory from June 1979 through August 1979. It was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials;" Task 630201, "Toxicology of Propellants and Materials;" Work Unit 63020104, "Teratogenic Screening of Air Force Chemicals." It was performed as a Summer Faculty Research Project by Dr. Lyng.

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INTRODUCTION

As more women enter jobs formerly held by men there exists the possibility that exposure to certain chemicals may cause birth defects. Generally, birth defects from exposure to chemicals would occur if exposure took place during the first half of pregnancy when most women would still be working.

Teratogenic potential is determined by animal testing. One of the difficulties with animal tests is that no single species can serve as an adequate indicator for all chemicals. As a result it is frequently necessary to test the same chemical in more than one species. This project was designed to determine the usefulness of ICR mice for testing teratogenic potential. The missile propellant hydrazine was selected for testing in the mouse because some information on its activity in other species is known.

Hydrazine is a slightly basic, polar compound that is a strong reducing agent. The toxicity of hydrazine has long been established. Exposure to hydrazine can lead to skin rashes, fatty liver, blood disorders and tumor induction (NIOSH, 1978). Less is known about the teratogenic effects of hydrazine. If embryos of the South African Clawed toad (Xenopus laevis) are exposed before neurulation, abnormal development occurs (Greenhouse, 1976). Hydrazine also causes defects in the neural tube and somites of chick embryos (Teland and Mulherkar, 1974).

Although tests in nonplacental organisms may give insight into possible mechanisms that disrupt development, they fail to show effects that may result from maternal metabolism. In placental mammals the effects of hydrazine have been studied in the rat. Lee and Aleyassine (1970) report that hydrazine is fetotoxic when administered during the last half of pregnancy and Keller et al. (manuscript in preparation) also report fetotoxicity when hydrazine is administered on days 6-15 of gestation with days 7-9 being the most sensitive.

METHODS AND MATERIALS

Sexually mature ICR mice were obtained from Harlan Industries. They were housed in plastic cages on wood chip bedding and received Purina Mouse Chow and water ad libitum. The temperature of the room was maintained at 70-76 F with a 12 hour light cycle. Four to five females were kept with each male. The females were checked for the presence of a vaginal plug early in the morning and again late in the afternoon. The day that a vaginal plug was found was designated as day zero of pregnancy. The pregnant mice were weighed daily.

Pregnant mice were assigned to a control group or to one of five experimental groups. All mice were injected intraperitoneally on days 6, 7, 8, and 9 of gestation. The control group received physiological saline. The experimental groups received hydrazine (Eastman, Rochester, New York) diluted with physiological saline at doses of 4, 12, 20, 30, and 40 mg/kg body weight.

On day 17 of gestation the pregnant females were killed and the fetuses removed by caesarian section. Using the methods from Olson and Back (1978) and Wilson and Warkany (1965), the number and position of each fetus was recorded, weighed and examined for abnormalities. Two thirds of the fetuses were preserved in Bouin's solution, sectioned with a razor blade and examined for soft tissue anomalies. One third of the fetuses were preserved in absolute ethanol, eviscerated, cleared in potassium hydroxide, stained with Alizarin Red S and examined for skeletal anomalies.

The Students T test was used to test for statistical significance on body weights, implantations per female, viable fetuses per litter, and resorptions per litter. The significance of the frequency of soft tissue and skeletal abnormalities was determined by the Fisher Exact Test using the litter as the experimental unit.

RESULTS

The effects of hydrazine on the number of implantations per female, viable fetuses per litter, and resorptions per litter are not apparent until the concentration exceeds 20 mg/kg body weight. There is no significant difference in the mean implantations per female receiving saline and the means of those mice receiving 4, 12, or 20 mg/kg hydrazine (Table 1). The same is true for the means of viable fetuses per litter and resorptions per litter. At 30 and 40 mg/kg, however, hydrazine is fetotoxic at the early stages of gestation. At a concentration of 30 mg/kg, only one female out of 30 produced a litter. At 40 mg/kg, none of 21 females produced a litter. Four of these 21 females died while there were no pregnant female deaths at any of the other concentrations tested.

TABLE 1

EFFECT OF HYDRAZINE ON THE MEAN NUMBER OF IMPLANTATIONS, VIABLE FETUSES AND RESORPTIONS

		Hydrazine		
	Saline	4 mg/kg	12 mg/kg	20 mg/kg
Number of Litters Examined	16	16	9	13
Implants/Female Mean ± SE	11.44±0.40	11.86±0.46	12.11±0.98	10.54±0.83
Viable Fetuses per Litter Mean ± SE	10.94±0.55	10.75±0.60	11.67±0.97	9.92±0.89
Resorptions per Litter Mean ± SE	0.50±0.26	1.13±0.43	0.44±0.24	0.62±0.24

The frequency of soft tissue abnormalities is presented in Table 2. Although there was an increasing frequency of soft tissue anomalies as the dose was increased, the difference between the saline treatment and the hydrazine treatment at 4 or 12 mg/kg is not significant ($p \leq 0.05$). The difference between saline and 20 mg/kg is significant ($p \leq 0.01$). At 20 mg/kg the largest number of litters and the most fetuses were affected. Exencephaly and hydronephrosis were the most frequently produced soft tissue anomalies.

TABLE 2

EFFECT OF HYDRAZINE ON THE FREQUENCY OF SOFT TISSUE ABNORMALITIES

		Hydrazine			
	Saline	4 mg/kg	12 mg/kg	20 mg/kg	30 mg/kg
Number of Litters (Fetuses) Examined	16(117)	16(115)	9(73)	13(91)	1(8)
Exencephaly	1(1)	2(2)		4(4)	1(1)
Meningo-encephalocoel	1(1)				
Anopthalmia				1(1)	
Microopthalmia		1(1)		1(1)	
Hydronephrosis		1(1)	1(1)	5(7)	1(1)
Hypoplasia of Teste			1(1)		
Undescended Teste			1(1)		
Enlarged Bladder	1(1)		1(1)		
Cleft Palate				1(1)	
Total Litters with Anomalies	3	4	4	8[a.]	1
Percent of Litters with Anomalies	19	25	44	62	100

a. Significant difference ($p \leq 0.01$) from saline control

The results of the examination for skeletal abnormalities show no significant difference ($p < 0.05$) between the control and those receiving 4 mg/kg (Table 3). However, all of the litters from females receiving 12 or 20 mg/kg had skeletal malformations and the differences between these treatments and the control are significant ($p < 0.01$). The one litter produced after treatment with 30 mg/kg also had skeletal abnormalities. By far the most prevalent abnormality was supernumerary ribs with two occurrences of short ribs and one with a bipartite vertebral centrum.

TABLE 3

EFFECT OF HYDRAZINE ON THE FREQUENCY OF SKELETAL ABNORMALITIES

		Hydrazine			
	Saline	4 mg/kg	12 mg/kg	20 mg/kg	30 mg/kg
Number of Litters (Fetuses Examined)	16(58)	16(57)	3(36)	13(38)	1(3)
Supernumerary Ribs	2(2)	1(1)	9(21)	13(31)	1(1)
Short Ribs	1(1)	1(1)			
Bipartite Centrum				1(1)	
Total Litters with Anomalies	3	2	9[a.]	13[a.]	1
Percent Litters with Anomaly	19	12	100	100	100

a. Significant difference ($p \leq 0.01$) from saline control

Hydrazine also affected the weight gain of pregnant mice. During the period when injections were given, days 6 through 9, mice receiving 12 and 20 mg/kg had the slowest rate of gain (Figure 1). From day 9 through day 13 these mice had the fastest rate of gain. In contrast, the mice receiving saline and 4 mg/kg showed essentially the same rate of gain from day 5 through day 13. On days 13 through 17 all of the mice had a similar rate of weight gain. Those mice showing the lowest rate of weight gain during the injection period also had the highest number of anomalies in their offspring. The one litter produced from the group receiving 30 mg/kg is not graphed and no litters were produced when 40 mg/kg was used.

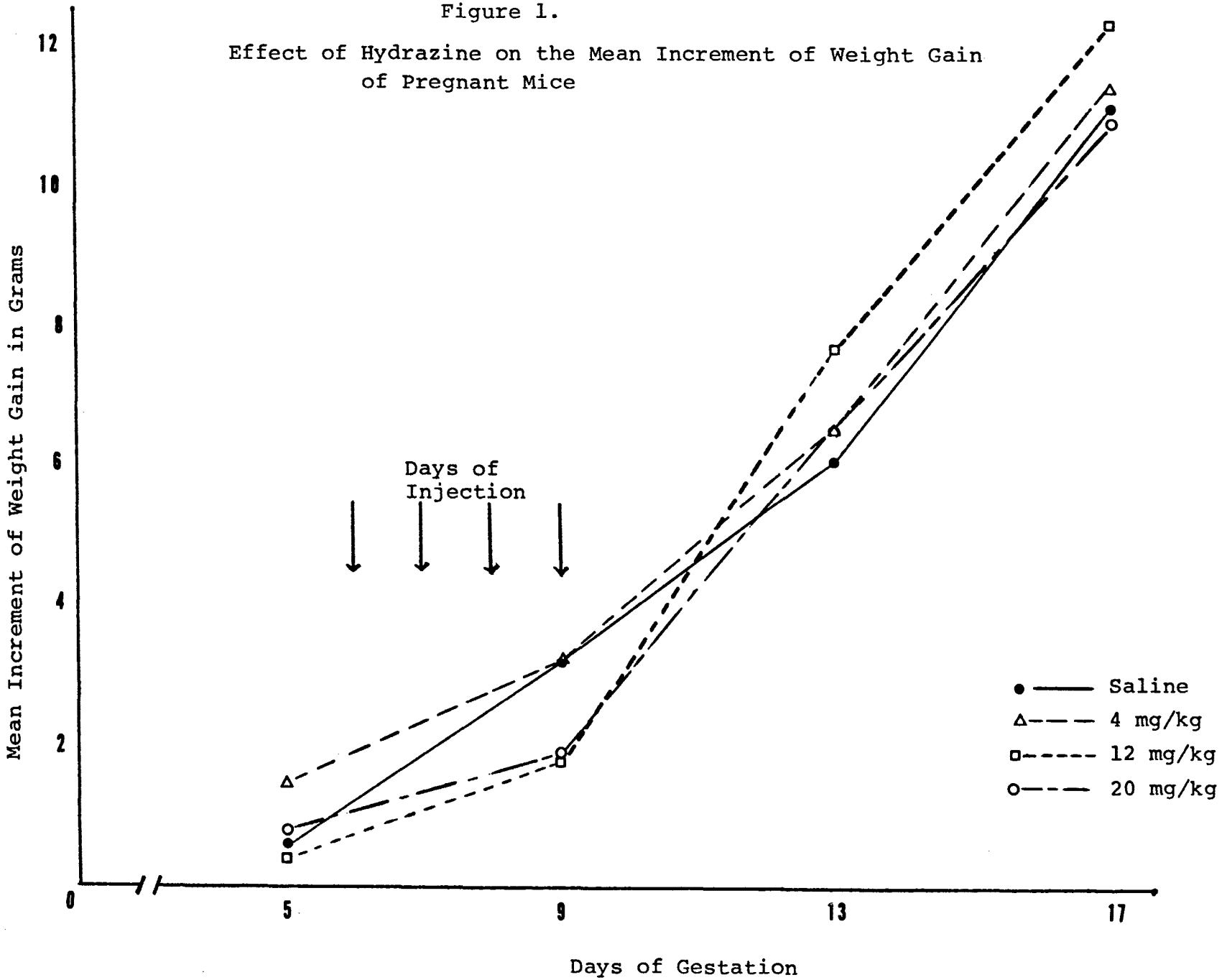
The fetal weights (mean $\pm$ S.E.) of the control and those receiving 4 mg/kg were identical at 1.05 ± 0.01 g. The mean weight of those given 12 mg/kg was 0.97 ± 0.01 g and those receiving 20 mg/kg was 0.98 ± 0.02. Only those from the 12 mg/kg treatment were significantly different from the control.

DISCUSSION

At concentrations of 20 mg/kg or lower the major effect of hydrazine was skeletal and soft tissue abnormalities. Skeletal abnormalities were

Figure 1.

Effect of Hydrazine on the Mean Increment of Weight Gain of Pregnant Mice



produced more frequently and at a lower concentration than were soft tissue abnormalities. There is a dose response to hydrazine. Skeletal abnormalities were the most sensitive and occurred at 12 mg/kg. Skeletal abnormalities increased at 20 mg/kg and soft tissue abnormalities also increased to a significant level at 20 mg/kg. Thus, abnormalities of development occurred before the concentration of hydrazine was toxic to the fetus. Toxicity was not present until a dose of 30 mg/kg was given. This concentration was very toxic to the fetus as only 1 litter from 30 pregnant mice was produced. Perhaps a dose response of fetal toxicity would be found if concentrations between 20 and 30 mg/kg were tested. In the rat there is a dose response to fetal toxicity in a range of concentrations that had no increase in skeletal or soft tissue abnormalities.

The data reported on fetal weights are hard to interpret. The only treatment which showed a significant decrease in fetal weight was 12 mg/kg. This dose produced skeletal abnormalities but the mean fetal weight at the higher dose of 20 mg/kg, which produced both skeletal and soft tissue abnormalities, was not significantly different from the control. It seems likely that if hydrazine affects fetal weight at 12 mg/kg it would also affect fetal weight at 20 mg/kg. Keller et al. (in preparation) reports a reduction in fetal weight of rats treated with 5 mg/kg hydrazine on days 6-15 of gestation.

A possible explanation for the differences in the response of mice and rats to hydrazine lies in the higher resistance of mice to the toxic effects of hydrazine. The LD_{50} for mice is 163 mg/kg and for rats it is 76 mg/kg when injected intraperitoneally (NIOSH, 1976). Even when mice and rats are given doses in roughly the same proportion of the LD_{50} dose (12 mg/kg for mice and 5 mg/kg for rats) skeletal abnormalities without fetal toxicity appear in mice and fetal toxicity without skeletal or soft tissue abnormalities appears in rats. This suggests that the mouse fetus, like the adult, is more resistant to the toxic effects of hydrazine but more susceptible to the teratogenic effects of this chemical.

The ICR strain of mice can be used as an additional species for teratogenic testing. Although the fetuses are smaller than rat fetuses, no difficulty was encountered in making or examining razor blade sections. Clearing in potassium hydroxide and staining with Alizarin Red S works well and takes less time than a rat fetus due to the smaller size. These mice breed well in the laboratory and no difficulties in handling were encountered.

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